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Baroreflex sensitivity in the elderly: influence of age, breathing and spectral methods

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A B S T R A C T

Baroreflex sensitivity (BRS) has been proposed as a diagnostic parameter for neurological disorders and as a survival-prognosis parameter in diabetic and cardiac patients. Therefore reference values and the reproducibility of BRS were assessed, taking into account the possible influence of age, gender, test conditions and some analysis variants. Healthy subjects ($n = 191$) were randomly selected from the 50–75-year-old general population (the Hoorn Study). Variations in blood pressure and heart rate were recorded non-invasively during three breathing modes: spontaneous (3 min), slow metronome (1 min; 6 breaths/min = 0.1 Hz) and fast metronome (1 min; 15 breaths/min = 0.25 Hz), all in a supine position. From these recordings, BRS was assessed as the transfer gain between changes in blood pressure and heart period, and as the α coefficient. BRS values ranged from 5.0 to 8.9 ms·mmHg^{-1}. Slow metronome breathing resulted in higher BRS values than fast breathing, while during spontaneous breathing BRS in the low-frequency band was lower than that in the high-frequency band (respiratory origin). BRS values decreased with lower coherence criteria. BRS-α was significantly higher than BRS-gain. While regression analysis showed no gender differences, BRS decreased with age. Therefore age-specific reference values were calculated. The reproducibility of BRS values was in general moderate, with reliability coefficients ranging from 43 to 81% and coefficients of variation ranging from 34 to 59%. In conclusion, this study shows age, breathing mode, frequency and coherence threshold to affect measures of BRS. Therefore these factors should be considered in clinical studies; appropriate reference values are given.

INTRODUCTION

The baroreceptor heart rate reflex, or baroreflex, is the fastest blood pressure buffering mechanism. In reaction to variations in blood pressure, detected by stretch receptors located mainly in the aortic arch and the carotid sinus, the baroreflex modulates cardiac vagal and sympathetic outflow to the sinus node in the heart. This baroreflex control of heart rate can be quantified by baroreflex sensitivity (BRS), which represents the amount of change in heart rate attributable to changes in systolic blood pressure.

BRS has been proposed as a new parameter to assess autonomic dysfunction in subjects with diabetes [1–4], in addition to or even as a substitute for the standard cardiovascular autonomic function tests (i.e. the deep

Key words: aging, autonomic nervous system, baroreflex, reference values, reproducibility.

Abbreviations: BRS, baroreflex sensitivity; CV, coefficient of variation; HF, high-frequency; LF, low-frequency; OGTT, oral glucose tolerance test; RC, reliability coefficient.

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breathing test, the lying-to-standing test, the Valsalva manoeuvre and the sustained handgrip test [3,5,6]). Besides its application in the assessment of cardiovascular autonomic function in diabetic patients, BRS has been introduced in cardiology for risk stratification after myocardial infarction [7,8]. However, reference values of BRS have not been assessed for a well-documented general population, and data on reproducibility are scarce, and have not yet been assessed for subjects of advanced age.

The classic procedure for assessing the sensitivity of the baroreceptor heart rate reflex involves the measurement of intra-arterial blood pressure and reflex heart period changes caused by an intravenous bolus injection of phenylephrine [9]. Non-invasive methods to assess BRS are now available, based on measurements of finger arterial blood pressure (Finapres®) and the joint analysis of spontaneous coherent changes in blood pressure and heart rate [10–13]. Three methods are the most common: the sequence technique [14], which is a time-domain method, and two frequency-domain methods: the spectral technique to assess the so-called α coefficient [10], and the cross-spectral technique to estimate the gain of the transfer function between changes in blood pressure and heart period [12,13,15,16]. In the present study we have used the two frequency-domain methods.

All three methods are based on the assumption that changes in heart period are driven by independent (systolic) changes in blood pressure through the baroreflex. It is commonly thought that the coherence, which corresponds to the correlation coefficient in the time-domain analysis, reflects this coupling between blood pressure and heart rate. It is therefore the case that a threshold is imposed on the coherence to increase the specificity of the estimates of BRS. However, any choice of this threshold would be arbitrary, but becomes pertinent in cases of low BRS values.

The sequence technique, the α coefficient and the transfer function method all assess dynamic features of the baroreflex control of heart rate, and not its steady-state value. Since it is known that the gain of the transfer function changes with frequency [16], the choice of the frequency bandwidth will also have an influence on BRS estimation.

Breathing evokes highly coherent changes in blood pressure and heart rate, and metronome breathing allows this exogenous stimulation to be associated with bands of a particular frequency. On the other hand, breathing may also cause non-baroreceptor-mediated changes in heart rate [11,16].

In order to address all these possible influences on BRS, the present paper reports reference values for BRS in a population sample of 50–75-year-old subjects, estimated by the transfer function gain and the α coefficient, from recordings in the supine position, during spontaneous breathing as well as metronome breathing at 6 or 15 breaths per min. Furthermore, the impact of the coherence criterium and the reproducibility of BRS are addressed.

METHODS

Study population

For the present analyses, data were obtained from the Hoorn Study, in which 2484 Caucasian men and women, born between 1914 and 1940, participated in 1989 and 1990. The study population and research design have been described in detail previously [17]. The study was originally conducted to assess the prevalence of glucose intolerance and diabetes-related disorders, in particular cardiovascular diseases. Therefore, a 75 g oral glucose tolerance test (OGTT) was performed. An age-, gender- and glucose-tolerance-stratified sample (708 subjects) was invited for a second OGTT within 3–5 weeks, and subjects were requested to undergo an extensive physical examination, including autonomic function tests, on another day. Of this sample, 631 (89%) participated in the study. Subjects were classified according to WHO criteria, based on the mean values of two OGTTs [18]. For this reference value study, only subjects with a normal glucose tolerance were included. Of the 288 subjects with a normal glucose tolerance, those with hypertension or prevalent self-reported cardiovascular or neurological diseases were excluded, resulting in a study population of 191 healthy subjects.

Reasons for exclusion were: a self-reported history of neurological disease (five subjects), self-reported chronic obstructive pulmonary disease (four subjects) and a history of cardiovascular disease (40 subjects), as assessed by means of a Dutch translation of the London School of Hygiene and Tropical Medicine questionnaire [19]. Blood pressure was measured twice before the start of the OGTT, on both occasions by means of a random zero mercury sphygmomanometer (Hawksley-Gelman). Exclusion on the basis of hypertension was defined as current treatment with anti-hypertensive drugs (49 subjects), mean systolic blood pressure over 160 mmHg and/or mean diastolic blood pressure over 95 mmHg (36 subjects).

Individual data were missing for the following reasons: the test schedule was not completed, the quality of the data was insufficient for processing (a poor blood pressure signal or arrhythmias), or more than 10% non-sinus beats in the total number of recorded beats.

Of the 631 initial subjects, 43 were invited to participate in a reproducibility study. Of these, 39 subjects responded, and were studied for the second time within 3 weeks.

The study protocol was approved by the Ethics Committee of the Academic Hospital of the Vrije

Universiteit. All study participants gave their informed consent.

Autonomic function tests

Participants were asked to refrain from smoking and drinking coffee for 2 h prior to the assessment of cardiovascular autonomic function. Tests took place between 08.30 and 16.00 hours at least 1 h after a light meal. Subjects rested supine in a quiet ambience, with a room temperature of between 19 and 22 °C. Tests were performed using three frequency-determined breathing modes: (1) slow breathing for 1 min at 6 breaths per min; (2) fast breathing for 1 min at 15 breaths per min; and (3) spontaneous breathing for 3 min. The correct frequency of breathing (6 or 15 breaths per min) was controlled and dictated by oral and visual instructions of the investigator, who in turn followed beeps generated by the data-acquisition program. When off-line spectral analysis of the systolic blood pressure data showed a clearly recognizable peak shifted from the expected frequency of breathing (margins of ± 0.02 Hz), the measurements were discarded. After each test a resting period of 1 min was allowed, to prevent an influence of the previous test conditions.

During the tests, heart rate and blood pressure were recorded continuously on a PC-based data-acquisition system. RR intervals were obtained from a bipolar ECG chest lead by a hardware QRS detector with an accuracy of 1 ms. Blood pressure was recorded continuously, using the Finapres® method (Finger Arterial Blood Pressure; Ohmeda BP2000). Finger arterial pressure recordings were sampled digitally at 200 Hz, and subsequently processed. First the blood pressure signal was low-pass-filtered, after which it was down-sampled (100 Hz). For each heart beat, values of systolic blood pressure were obtained from this processed blood pressure signal by means of an automatic procedure, which was verified by visual inspection.

Computation of parameters

BRS was computed as (1) the transfer gain of blood pressure and heart period changes, and (2) the α coefficient, a ratio of the changes in heart period and blood pressure. Both methods will be explained below. For more detailed information on the computation of the spectral powers of RR intervals and systolic blood pressure within the particular frequency bands as used for both methods of computation, we refer the reader to a paper by TenVoorde et al. [20].

Transfer function gain: when we consider the baroreceptor heart rate reflex as a simple linear single-input single-output system, BRS is the change in heart period (output) that is caused directly by a unit change of systolic blood pressure (input), in $ms \cdot mmHg^{-1}$ [10,12,16,21]. BRS may then be estimated as the gain of the transfer function between systolic blood pressure and heart period (see Appendix 1). An additional function can be derived from systolic blood pressure (input), heart period (output) and cross-spectra between the two: the squared coherence (γ^2). This is a normalized function, which gives the amount of RR-interval variance that is linearly explained by the blood pressure variance. The squared coherence function has a value between 0 and 1: the higher this value, the higher the coupling between variations in blood pressure and heart rate.

Transfer gain and coherence are both functions of frequency (Figure 1). BRS values therefore are defined for bands of a particular frequency as the average of the gain values for those frequency components having a squared coherence value greater than 0.5, as is indicated by the bold parts of the curves of the transfer functions in Figure 1 (right-hand panels). These frequency bands (horizontal arrows in Figure 1) were: for the spontaneous breathing test a low-frequency (LF) band (0.04–0.12 Hz) and a high-frequency (HF) band (0.12–0.40 Hz); for the slow breathing test (6 breaths/min = 0.10 Hz) the 0.05–0.15 Hz frequency band; and for the fast breathing test (15 breaths/min = 0.25 Hz) the 0.20–0.30 Hz frequency band. BRS values for these particular breathing rates and frequency bands are denoted as: BRS-gain-LF, BRS-gain-HF, BRS-gain-6/min and BRS-gain-15/min. It is essential to keep in mind that, of these four BRS values, three are computed from variations in blood pressure and heart rate caused directly by breathing movements. Only BRS-gain-LF is based on spontaneous oscillations in blood pressure and heart rate as they appear in the frequency band around 0.1 Hz.

The squared coherence threshold of 0.5, although often applied [13,16], is rather arbitrary. We therefore assessed the influence of the squared coherence criterium on the BRS values by applying different thresholds of 0.7, 0.5, 0.3 and 0.0. Both formulae of gain and coherence functions and their relationship are given in Appendix 1 [13,15,16,21,22].

The α coefficient is defined as the square root of the ratio of the spectral powers of RR interval and systolic blood pressure within a band of a particular frequency. The ratio was only calculated for those points in the spectrum with a coherence greater than 0.5 [10,23]. Thus, in contrast with the BRS gain, BRS-α does not include the coherence, but the coherence is only used as a cut-off value above which it is valid to compute the BRS (Appendix 1). The α coefficient was only calculated from the spontaneous breathing recordings for both the LF and HF bands, as is generally done, and is denoted as BRS-α-LF and BRS-α-HF.

Statistical analysis

A normal distribution of all BRS values was obtained by taking the natural logarithm. For the presentation of the geometric means, 95% confidence intervals and per-

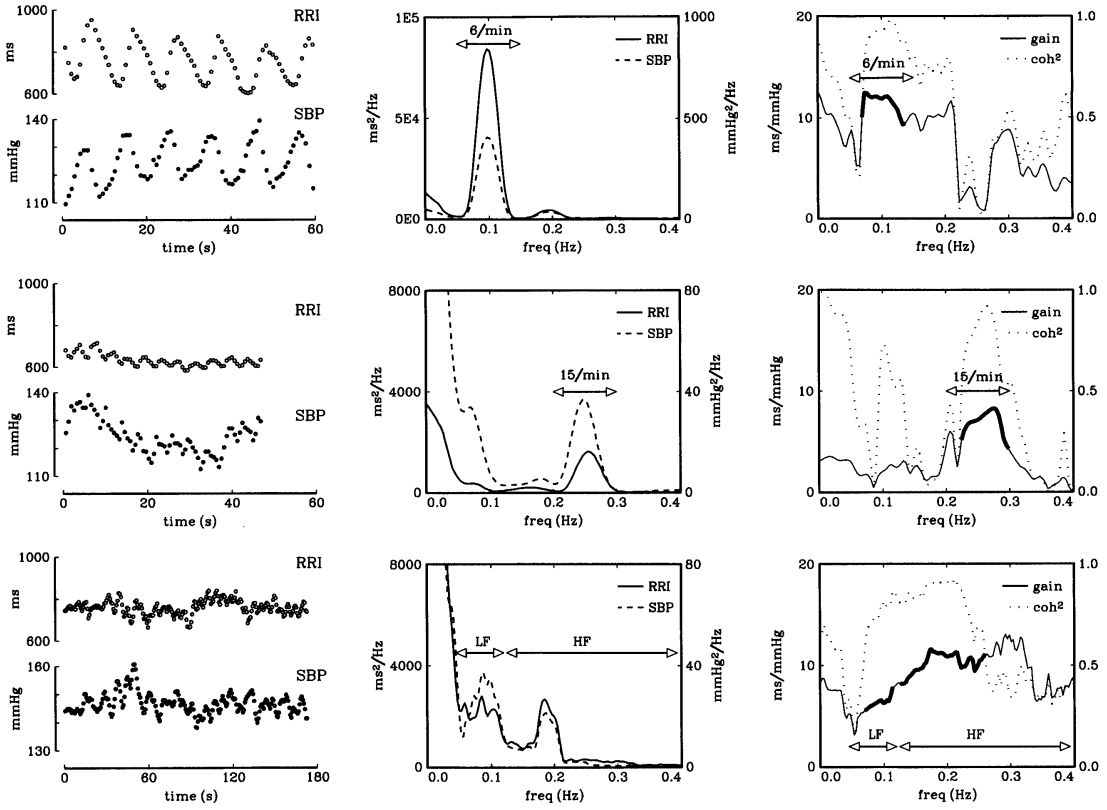

Figure 1 An example of cross-spectral analysis of blood pressure and heart rate and computation of BRS for a healthy woman (57 years old)

From left to right: left-hand panels show the beat-to-beat series of RR interval (RRI; ms) and systolic blood pressure (SBP; mmHg); middle panels show the amount of variance in RRI and SBP as a function of frequency, i.e. power spectra; right-hand panels show the transfer gain between SBP and RRI and squared coherence as a function of frequency. From top to bottom: upper panels show the results of the slow metronome breathing test; middle panels show the results of the fast metronome breathing test; lower panels show the results of the spontaneous breathing test. For each breathing mode, different frequency bands are defined as indicated by the horizontal arrows. Within these frequency bands BRS-gain values are computed as the average transfer gain, including only those spectral components meeting the squared coherence criterium (bold lines).

Table 1 Characteristics of the healthy elderly study population ($n = 191$)

HDL, high-density lipoprotein. Values are means ± S.D.

Variable	Value
Gender	
Men	98
Women	93
Age (years)	62.6 ± 7.4
Body mass index (kg/m^2)	25.5 ± 3.0
Fasting plasma glucose (mmol/l)	5.4 ± 0.5
Total cholesterol (mmol/l)	6.6 ± 1.1
HDL cholesterol (mmol/l)	1.4 ± 0.4
Triglycerides (mmol/l)	1.5 ± 0.7
Mean RR interval (ms)	
Supine	65 ± 135
Standing	847 ± 116
Diastolic blood pressure (mmHg)	78.4 ± 7.4
Systolic blood pressure (mmHg)	128.1 ± 12.8

centiles, ln values were back-transformed. To evaluate the influence of breathing mode on BRS values, paired *t*-tests were performed. Two regression models were used to assess the possible influence of age and gender: (1) $\ln(\mathrm{BRS}) = \alpha + \beta_1 \cdot \mathrm{age} + \beta_2 \cdot \mathrm{gender}$, and (2) $\ln(\mathrm{BRS}) = \alpha + \beta_1 \cdot \mathrm{age}$, with age in decades and gender as a dichotomous variable (0 = male and 1 = female). Transformation of BRS was indicated again, now to obtain normally distributed residuals. Reference values, defined as the 90% prediction interval for individual predictions, were computed from the estimated linear regression parameters, thus optimally using the available measurements [24]. The formulae used are presented in Appendix 2.

For the population for which duplicate measurements were available, the reliability coefficient (RC) and the coefficient of variation (CV) were calculated by analysis of variance to obtain test/retest reliability [25]. Thus:

$$\mathrm{RC} = \mathrm{S.D.}^2_{\mathrm{between}} / (\mathrm{S.D.}^2_{\mathrm{between}} + \mathrm{S.D.}^2_{\mathrm{within}}) \cdot 100\,\%$$

with high values of RC representing good reproducibility; and:

$$CV = S.D._{within}/mean_{both\ measurements} \cdot 100\ \%$$

with low values representing good reproducibility.

RESULTS

Reference values

The mean age of subjects in the reference value study was 62.6 ± 7.4 years (Table 1). As can be seen from Tables 2 and 3, the BRS values differed significantly between the various breathing modes, frequency bands and squared coherence criteria, ranging from 5.0 to 8.9 ms · mmHg^{-1}. BRS-gain-6/min and BRS-α-HF were higher than any of the other BRS values measured, and BRS-gain-LF was the lowest (Table 2). The α coefficients were, in both the LF and HF bands, higher than the respective transfer function gains (Table 2).

BRS values increased with increasing coherence criteria (Table 3), with the greatest increases for BRS-gain-LF and BRS-gain-HF, i.e. more than 2 ms · mmHg^{-1}. The observed coherence between variations in blood pressure and heart period was significantly lower for the spontaneous breathing data (Table 3, final column). Mean squared coherence over the HF band was even below 0.5. Applying a higher coherence criterium of 0.7 instead of 0.5 (Table 3, first column) led to the exclusion of 27 persons for the spontaneous breathing tests, but none for the metronomic breathing tests. The excluded persons were on average 2 years older than those for whom the BRS could be computed, but they had a comparable average systolic blood pressure, comparable fasting blood glucose, comparable HbA_{1c} (glycated haemoglobin) and comparable lipid profiles.

After adjustment for age, the regression analysis showed no clear consistent differences between males and females. Therefore no gender-specific reference values are presented. BRS estimates for the slow metronome test and the spontaneous breathing test decreased significantly with increasing age, ranging from 0.1 to 0.4 ms · mmHg^{-1} per 10 years. For fast metronome breathing, the magnitude of the estimate of the regression coefficient was similar, but it did not attain statistical significance. Reference values for each BRS value for six different ages are given in Table 4.

Table 2 BRS computed as transfer gain and α coefficient estimated from recordings made under different breathing conditions in the supine position, in a healthy population aged 50–75 years

The squared coherence criterium was 0.5. CI, confidence interval. Frequencies: 6/min, 6 breaths/min (0.1 Hz); 15/min, 15 breaths/min (0.25 Hz); LF, 0.04–0.12 Hz; HF, 0.12–0.40 Hz. Significance of differences (*t*-tests): $^*P < 0.05$ for metronomic compared with spontaneous breathing; †$P < 0.05$ for LF compared with HF; ‡$P < 0.05$ for BRS-gain compared with α coefficient.

	BRS (ms · mmHg^{-1})		
	Geometric mean	95% CI	*n*
Metronome breathing			
BRS-gain-6/min	8.5*†	7.9 to 9.2	167
BRS-gain-15/min	6.6*	5.9 to 7.4	169
Spontaneous breathing			
BRS-gain-LF	6.2†‡	5.6 to 6.8	160
BRS-α-LF	6.6†	6.0 to 7.3	149
BRS-gain-HF	7.5‡	6.7 to 8.3	160
BRS-α-HF	8.6	7.7 to 9.6	157

Reproducibility

The mean, RC and CV were calculated for all BRS values (Table 5). In general, the reproducibility of BRS values was poor to moderate, with RCs ranging

Table 3 Geometric means of BRS computed as the transfer-function gain with multiple coherence criteria for the three breathing modes in a healthy population aged 50–75 years

For the case that no squared coherence criterium was applied, i.e. 0.0, the observed squared coherence is given. CI, confidence interval. Frequencies: 6/min, 6 breaths/min (0.1 Hz); 15/min, 15 breaths/min (0.25 Hz); LF, 0.04–0.12 Hz; HF, 0.12–0.40 Hz.

	BRS (ms · mmHg^{-1})				
Squared coherence criteria ...	0.7	0.5	0.3	0.0	Observed squared coherence [95% CI]
Metronome breathing					
BRS-gain-6/min	8.9	8.5	8.2	7.8	0.73 [0.71 to 0.75]
BRS-gain-15/min	6.9	6.6	6.4	6.0	0.69 [0.67 to 0.71]
Spontaneous breathing					
BRS-gain-LF	6.9	6.2	5.7	5.0	0.52 [0.50 to 0.54]
BRS-gain-HF	7.9	7.5	6.9	5.6	0.43 [0.41 to 0.45]

Table 4 Reference values for BRS as a function of age

Given are the 90% prediction intervals for individuals, as estimated from the reference population aged 50–75 years (n = 191). The squared coherence criterium was 0.5. Frequencies: 6/min, 6 breaths/min (0.1 Hz); 15/min, 15 breaths/min (0.25 Hz); LF, 0.04–0.12 Hz; HF, 0.12–0.40 Hz.

		BRS (ms · mmHg^{-1})					
	Age (years) ...	50	55	60	65	70	75
Metronome breathing							
BRS-gain-6/min	5%	4.7	4.3	4.0	3.6	3.3	3.0
	95%	24.3	22.0	20.0	18.3	16.7	15.3
BRS-gain-15/min	5%	1.8 for all ages					
	95%	22.7 for all ages					
Spontaneous breathing							
BRS-gain-LF	5%	3.4	3.0	2.6	2.2	1.9	1.6
	95%	23.4	20.0	17.2	14.8	12.8	11.1
BRS-α-LF	5%	3.5	3.0	2.7	2.3	2.0	1.8
	95%	24.9	21.6	18.8	16.5	14.4	12.7
BRS-gain-HF	5%	3.9	3.3	2.8	2.3	1.9	1.6
	95%	35.0	29.1	24.3	20.4	17.1	14.5
BRS-α-HF	5%	4.8	4.0	3.3	2.7	2.2	1.8
	95%	41.8	34.0	27.7	22.6	18.5	15.2

Table 5 Reproducibility of BRS in a 50–75-year-old general population, expressed as RC and CV

The squared coherence criterium was 0.5. Note that this population was not selected on the basis of health parameters. Frequencies: 6/min, 6 breaths/min (0.1 Hz); 15/min, 15 breaths/min (0.25 Hz); LF, 0.04–0.12 Hz; HF, 0.12–0.40 Hz.

	Mean BRS (ms · mmHg^{-1})	RC (%)	CV (%)	n
Metronome breathing				
BRS-gain-6/min	7.7	43	34	36
BRS-gain-15/min	6.2	55	59	27
Spontaneous breathing				
BRS-gain-LF	5.1	48	43	28
BRS-α-LF	6.0	50	39	19
BRS-gain-HF	5.5	71	50	27
BRS-α-HF	6.5	81	42	26

from 43 to 81% and CVs ranging from 34 to 59%. BRS-α-HF had the best reproducibility, with an RC of 81%, and BRS-gain-6/min had the best (lowest) CV of 34%.

DISCUSSION

The present study reports the 5th and 95th percentiles, age-specific values for BRS and its reproducibility in a healthy 50–75-year-old population. We observed differences in BRS values between the various breathing modes and frequency bands, and a dependence on coherence criteria. The observed geometric means varied between 5.0 and 8.9 ms · mmHg^{-1}, thus stressing the importance of standardization and the use of appropriate reference values.

Reference group

The reference group was taken from a random sample of the general population. Only subjects with a normal glucose tolerance, no cardiovascular diseases, no hypertension and no neurological diseases were selected, because these conditions are known to lower BRS. As we did not exclude subjects with subclinical disease, as may be assessed by more advanced testing, we selected a population that was representative of the general healthy population.

The WHO definition for hypertension, i.e. blood pressure of 160/95 mmHg, was used [26]. Currently, values between 160 and 140 mmHg systolic and 95 and 90 mmHg diastolic are referred to as mild hypertension, and this condition is known to be associated with lower BRS [23]. Therefore we repeated the analyses with the exclusion of subjects with mild hypertension (results not shown). Although the average age of the 37 excluded persons was higher than the age of the remaining 154 persons [66.4 (S.D. 7.3) versus 61.7 (7.1) years], the estimated decrease with age as assessed by linear regression and the estimated reference values remained essentially the same. Furthermore, including systolic blood pressure as a continuous variable in the linear regression analysis showed no significant association between systolic blood pressure and measures of BRS.

Age

The finding that BRS diminishes with age is consistent with previous findings [23,27]. The present study, however, extends this finding in a population sample of more advanced age. One previous study by James et al. [28] did not observe age-related differences in an elderly population aged 60–81 years. However, this might have been due to the slightly greater age and the 4-fold smaller size in comparison with our study population. Since BRS decreased with increasing age, age-specific reference values are recommended. Observed differences in cut-off points were substantial, e.g. 3.4 $ms \cdot mmHg^{-1}$ for a person of 50 years to 1.6 $ms \cdot mmHg^{-1}$ for a person of 75 years of age for BRS-gain-LF in the supine position. La Rovere et al. [8], in their study addressing the prognostic value of BRS for survival in patients following myocardial infarction, used the phenylephrine method and a cut-off value of 3.0 $ms \cdot mmHg^{-1}$ to define the high-risk group. Our reference values are close to this value of 3.0 $ms \cdot mmHg^{-1}$, supporting the validity of the definition of abnormality on a statistical basis. Linden and Diehl [29] also reported reference values for BRS; unfortunately, they measured RR interval variation with the Finapres device and not with an ECG, making it less precise. Further, the reported values were mean values and not really reference values.

Gender

No differences were found between men and women. In middle-aged subjects, Huikuri et al. [30] reported gender differences in BRS: men having higher values (10.5 ± 4.6 $ms \cdot mmHg^{-1}$; $n = 188$) than women (8.0 ± 4.6 $ms \cdot mmHg^{-1}$; $n = 186$). A possible explanation might be the slightly younger age of the study population. Moreover, their study population was approximately twice the size of our study population, thus implying greater power to detect relatively small differences.

Methodological issues

There is no clear consensus on how to assess BRS non-invasively. Many different analysis methods and analysis parameters exist: time domain versus frequency domain, different spectral algorithms, and bands of different frequency. The dependence of BRS on several of these issues was the subject of investigation in the present study, and will be discussed in the following three sections. This section elaborates on some additional methodological issues.

We used slightly different frequency bands compared with the ESC Task Force recommendations for studies on heart rate variability [31] (note that BRS assessment was not part of these recommendations). We positioned the border frequency between the LF and HF bands at 0.12 Hz, whereas the Task Force on heart rate variability suggests 0.15 Hz. [31]. We did this because we think that this will give a better differentiation between respiration-induced variability (HF) and spontaneous oscillations (LF). The frequency of spontaneous respiration in the supine resting position often falls below 0.15 Hz. On the other hand, the effect of a small shift in this LF-to-HF border frequency on BRS estimation is expected to be rather small, since BRS is based on a ratio of heart rate to blood pressure powers, together with a coherence criterium.

For a true and unbiased estimate of BRS, it is assumed that variations in heart rate are secondary to changes in blood pressure, and thus mediated by the baroreflex. This does not entirely hold for respiratory-induced variations, as will be discussed in the next section. Nevertheless we decided to assess BRS from modulations in spontaneous breathing (HF band) as well as from variations induced by metronome breathing (6 and 15 breaths/min), firstly to estimate the impact of respiration and frequency on BRS. Secondly, a BRS estimate based on 1 min of metronome breathing at 6 breaths/min seems a clinically interesting autonomic function parameter, since from these differences in heart rate the so-called expiration/inspiration value (EI-value) can also be computed, which is one of the oldest and best standardized parameters used to quantify autonomic dysfunction in diabetes [5,32].

Finally, some comments on our computation of the α coefficient. Although the definition is not different from Pagani's original one [10], the computation of the underlying power spectra does differ. While Pagani used an autoregressive method, we used a periodogram-like method, a modified form of the Discrete Fourier Transform [20]. Both methods are valid ways to estimate the power spectral density function, although the autoregressive method is known to have a better frequency resolution, in particular for short recordings. However, in the case of the α coefficient, one applies the power over the selected frequency band, i.e. spectral components are summated, and this frequency resolution difference becomes irrelevant. Moreover, in a study on physiological data, the two power spectral density estimation methods did not give systematic differences in the BRS computation, and the reported differences were 10–20 times smaller than the observed inter-individual differences [33]. A final argument for computing the α coefficient from the Discrete Fourier Transform spectra in our study was the possibility of comparing both BRS-α and BRS-gain results in conjunction with the coherence criterion, which was an important goal of the present study, and which will be discussed in the next two sections.

Frequency dependence and respiration

It is generally accepted that variations in heart rate with frequencies above 0.15 Hz represent vagal function only,

while lower frequencies represent both vagal and sympathetic function [16,31]. Furthermore, the dynamic range of vagal, noradrenergic cardio-chronotropic control is also limited, with a cut-off frequency estimated at around 0.2 Hz in the supine position [15,16]. It is therefore not surprising that we found BRS values to depend on frequency: the three BRS-gain values computed from respiratory-induced fluctuations of 6/min, HF and 15/min are 8.5, 7.5 and 6.6 ms · mmHg^{-1} respectively, with the centre frequencies at which the BRS was computed being 0.10, 0.20 (mean spontaneous respiration rate for all subjects) and 0.25 Hz respectively.

BRS-gain-LF and BRS-gain-6/min are both computed within approximately the same frequency band around 0.1 Hz (see Figure 1, centre panels). However, BRS-gain-6/min results in a significantly higher BRS value: 8.5 compared with 6.2 ms · mmHg^{-1} (Table 2). There are two effects that probably explain this difference. First, a significantly lower coherence value was found for BRS-gain-LF ($\gamma^2 = 0.52$) compared with BRS-gain-6/min ($\gamma^2 = 0.73$) (Table 3, final column). This inherently results in a lower BRS value, as will be discussed in the section below. Secondly, the difference in origin between breathing modulations and the spontaneous existing so-called Mayer blood pressure waves (10 s waves; 0.1 Hz) [11,12,15] could also cause an overestimation of BRS. Breathing causes, besides baroreflex-mediated respiratory sinus arrhythmia, also non-baroreflex (though breathing-coherent) variations in heart rate, originating directly from the brain stem or evoked by lung stretch receptors. These non-baroreflex-mediated variations in heart rate will lead to an overestimation of BRS.

The two effects, i.e. the frequency dependency (resulting in lower values) and the overestimation due to respiration (resulting in higher values) may have a counteracting effect on BRS-HF compared with BRS-LF. The difference therefore may become small, but we found that BRS-gain-HF is still higher than BRS-gain-LF (7.5 and 6.2 ms · mmHg^{-1} respectively; $P < 0.05$), and the same holds for BRS-α-HF compared with BRS-α-LF (8.6 and 6.6 ms · mmHg^{-1} respectively; $P < 0.05$). In an earlier study of the effects of physical training on BRS, no significant differences were found between HF and LF α coefficients [10], while in a more recent study in conscious cats our results were indeed confirmed [34].

Coherence

Irrespective of the method of assessing BRS, be it the phenylephrine method [9], the sequence method [14], the spectral α method [10] or the cross-spectral transfer method [13], all rely on the assumption that observed modulations in RR intervals are driven essentially by the baroreflex in response to independent changes in systolic blood pressure. To increase the specificity of the BRS estimate, commonly a threshold is imposed on either the correlation (time-domain methods) or the coherence (frequency-domain methods). In general, a coherence threshold that is too high will be too selective and bias for instance reference values, in particular in an ill or elderly population in whom heart rate variability is already low. The coherence criterium of 0.7 in our study led to the exclusion of approx. 15% of the studied population, a group which appeared to be significantly older than those that were included, but nonetheless clearly healthy.

A second effect, which has been recognized previously [35,36], is the direct relationship between the height of the coherence (γ^2) and the transfer function gain (BRS) itself, in contrast with the α coefficient (Appendix 1). BRS estimated by cross-spectral analysis is equal to the square root of the power ratios (by definition the α coefficient) [14], scaled by the coherence. The latter factor compensates for non-coherent RR-interval variability, and will in practice always lead to lower BRS estimates compared with the straightforward power ratio ($= \alpha$ coefficient). Thus the coherence acts as a kind of weighing function for the transfer gain, while for the α coefficient it is only involved in selecting the RR intervals and values of systolic blood pressure on which the computation will be based. Our observations that the absolute values of the α coefficient were consistently higher than the transfer function gain parameters were in line with this. Furthermore, as a consequence, for the transfer gain it holds that the higher the imposed coherence threshold, the higher the BRS outcome, which is essentially shown in the present study.

Thirdly, as would be expected, metronome breathing significantly increased coherence between blood pressure and interval fluctuations. While the coherence threshold of 0.5 was not met for all spontaneous LF and HF BRS estimates, a coherence of 0.7 and higher was common for all metronome breathing segments. In conclusion, a squared coherence criterium of 0.5 seems appropriate for spontaneous breathing tests, whereas for metronomic breathing tests a higher squared coherence criteria of 0.7 can be applied.

Recording period

The minimal period of recording of blood pressure and heart rate data sets required to obtain reliable spectral estimates of BRS is in general an important matter of concern. First, a clear distinction has to be made between spontaneous breathing and metronome breathing data sets. Robbe et al. [13] used, and the ESC Task Force [31] recommended, 5 min of recording; however, this referred to data obtained during spontaneous breathing. In the case of metronome breathing at a particular frequency, the power of heart rate and blood pressure variability is importantly increased and concentrated in a relatively small frequency band in which the BRS was computed. The time series are also likely to become more stationary (spectral parameters vary less over time) compared with recordings during spontaneous breathing. Thus, in the

case of metronome breathing and of applying the respiration-evoked modulations in blood pressure and heart rate, much shorter recording periods will be sufficient to compute BRS. This seems to be confirmed by our data, since coherence values were significantly greater for the metronome breathing data (6/min) compared with the spontaneous breathing data (LF band). Secondly, the metronome breathing recordings should be kept as short as possible (in particular the slow deep breathing), in order to prevent hyperventilation, which will alter central modulations of heart rate and blood pressure control. A period of 1 min seems to be a reasonable compromise.

Reproducibility study

In general, reproducibility was rather poor. For example, for BRS-gain-LF and BRS-α-LF, the most applied non-invasive BRS values, the RC and CV were 48 % and 43 % for the transfer gain, and 50 % and 39 % for the α coefficient. The rather high CV values indicate that a single measurement is insufficient to characterize subjects. This poor reproducibility may be partly attributable to the advanced age of the subjects, leading to low BRS values, which directly results in higher CVs ($CV = S.D._{within}/mean_{both\ measurements} \cdot 100\,\%$). Reproducibility may be improved by standardizing the conditions during measurement, e.g. longer resting periods and carrying out the duplicate measurements at the same time of the day. However, the results presented here are more indicative of daily practice, and in that sense more valuable. However, clearly, a larger study confirming our findings on reproducibility will be necessary. In this context, the study by Herpin and Ragot [37] is worth mentioning, since they report, for BRS [cross-spectral analysis; mid-frequency band (0.066–0.127 Hz)], 1-week and 1-year reproducibility in 14 healthy subjects aged 23–51 years; RCs of 0.85 and 0.54 respectively were found.

Conclusions

From the present study, based on a carefully selected healthy sample of a general population, we conclude that non-invasively assessed BRS is still dependent on age even in the elderly, but is also, importantly, dependent on methodological choices. Of particular importance are: (1) the choice to apply either the α coefficient or the transfer-gain method; (2) the choice whether to use respiration-induced or spontaneous existing LF variability for the spectral analysis; (3) the choice of a band of certain frequency; and (4) the choice of coherence threshold. These conclusions emphasize the need for standardization before a further spread in clinical practice can be recommended. Since no international consensus has yet been reached, we have presented several age-specific reference values for several spectral methods, breathing modes and frequency bands.

One cannot expect clear recommendations from the current type of study alone as to which particular non-invasive method of BRS analysis should be used. Also, larger studies on reproducibility, determinants of autonomic function test outcomes [32] and predictive power are needed in order to reach a consensus for clinical use. Nevertheless, we may draw some useful guidelines from the data in the present study. The transfer-function method to compute BRS is on theoretical grounds preferable, since it truly is an estimate of reflex 'transfer', partly correcting for non-correlating noise sources, and additionally providing the phase relationship between changes in blood pressure and heart rate (results not shown). The α coefficient has the advantage of being conceptually simple, but always overestimates true transfer gain, although it comes close to BRS gain when coherence is high. Metronome breathing increases and concentrates blood pressure and heart rate power within a certain frequency band, therefore substantially increasing coherence, and hence allowing shorter recording periods. However, the trade-off will be a biased (overestimated) BRS due to respiration-induced, but non-baroreflex-mediated, variations in heart rate. Although it is a less specific baroreflex heart rate parameter, it still will be an autonomic nervous function parameter. BRS values depend on frequency. The advantages of assessing BRS from fluctuations during slow breathing (6/min) are the larger BRS values obtained at this frequency, the larger coherence and the low CV. This particularly becomes relevant in older and sicker patients. An additional practical advantage may be that a BRS assessment can be made from the same manoeuvre as the classical expiration–inspiration difference in RR intervals (Ewing). Consensus about what coherence criterion to apply seems crucial for both the transfer-gain and α-coefficient methods. It becomes less important, though, when metronome (slow) breathing is applied, since coherence is then sufficiently high.

In conclusion, age, breathing mode and breathing frequency all affect the observed BRS value. Therefore specific reference values for age, breathing mode and breathing frequency should be used.

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APPENDIX I

Transfer gain, α coefficient, squared coherence and their relationship

In the relationships below, the baroreceptor heart rate reflex is considered as a simple linear single-input/single-output system, where s denotes the input signal (systolic

blood pressure; SBP) and i denotes the output signal (RR interval; RRI), S_{ss} = power-spectrum SBP, S_{ii} = power-spectrum RRI, S_{si} = cross-spectrum SBP and RRI, and γ = coherence. The gain of the transfer function between SBP and RRI (BRS-gain) and the squared coherence are computed directly from the cross-spectrum and respective input/output spectrum [21] according to the following two equations:

$$\text{BRS-gain} = |S_{si}|/S_{ss} \quad \text{(A1.1)}$$

$$\gamma^2 = |S_{si}|^2/S_{ss} \cdot S_{ii} \quad \text{(A1.2)}$$

Eqn (A1.2) can be rewritten as:

$$|S_{si}|^2 = \gamma^2 \cdot S_{ss} \cdot S_{ii} \quad \text{(A1.3)}$$

Fill eqn (A1.3) into eqn (A1.1):

$$\text{BRS-gain} = \sqrt{\gamma^2} \cdot \sqrt{S_{ii}}/S_{ss} \quad \text{(A1.4)}$$

By definition, the α coefficient is the square root of the ratio of output and input spectrum [10]:

$$\text{BRS-}\alpha = \sqrt{S_{ii}}/S_{ss} \quad \text{(A1.5)}$$

From eqns (A1.4) and (A1.5), the direct relationship between the transfer function gain and the α coefficient follows:

$$\text{BRS-gain} = \sqrt{\gamma^2} \cdot \text{BRS-}\alpha \quad \text{(A1.6)}$$

APPENDIX 2

Reference values obtained from linear regression

The reference values were computed as the 90% prediction intervals [24]. These intervals are computed by means of linear regression, as follows. The general formula is:

$$90\%\ \text{interval} = \text{fitted value} \pm (t\text{-value}) \cdot \text{S.D.}$$

$$= \hat{y} \pm t s(\hat{y})$$

with fitted value:

$$\hat{y} = a + bx = \bar{y} + b(x - \bar{x}) \quad \text{(A2.2)}$$

and the standard error:

$$s(\hat{y}) = S_{yx}\sqrt{1 + \frac{1}{N} + \frac{(x-\bar{x})^2}{\Sigma(x_i - \bar{x})^2}} \quad \text{(A2.3)}$$

Given the standard error of the slope of the regression line, i.e.:

$$S.E.(b) = \frac{S_{yx}}{\sqrt{\Sigma(x_i - \bar{x})^2}} \quad \text{(A2.4)}$$

eqn (A2.3) can be rewritten as:

$$s^2(\hat{y}) = {s_{yx}}^2\left(1 + \frac{1}{N}\right) + (x - \bar{x})^2\,\text{S.E.}^2(b) \quad \text{(A2.5)}$$

and thus the standard error can be computed as:

$$s(\hat{y}) = \sqrt{{s_{yx}}^2\left(1 + \frac{1}{N}\right) + (x - \bar{x})^2\,\text{S.E.}^2(b)} \quad \text{(A2.6)}$$

the components of which are all readily available from the output of a statistical software package.

Example

The 5% and 95% limits of BRS from the slow metronome breathing test in the supine position for a 60-year-old person were computed using eqns (A2.2), (A2.6) and (A2.1). The intercept (a) = 3.294, the regression coefficient (b) = −0.185, the mean age (x) = 6.2378 decades, and the mean ln(BRS) (y) = 2.1419 are substituted into eqn (A2.2):

$$\hat{y} = 2.1419 + (-0.185)(6.0 - 6.2378) = 2.1859$$

Accordingly, the variance of the residuals (s^2_{xy}) = 0.239, the standard error of the regression coefficient S.E. (b) = 0.053 and N = 167 are substituted into eqn (A2.6):

$$s(\hat{y}) = \sqrt{(0.239)(1 + 1/167) + (6.0 - 6.2378)^2(0.053)^2} = \sqrt{0.2406} = 0.4904$$

With a t-value = 1.645 and the above estimated values substituted into eqn (A2.1), the 90% prediction interval becomes (in logarithmic values):

$$2.1859 \pm (1.645)(0.4904) = [1.375;\ 2.997]$$

Thus the 90% prediction interval is: $e^{1.375} = 4.0$ to $e^{2.997} = 20.0$ ms·mmHg^{-1}

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